GENERAL NOTES

Journal of the Lepidopterists' Society
53(4), 1999, 169–170

IS SPERMATOPHORE NUMBER A GOOD MEASURE OF MATING FREQUENCY IN FEMALE *CALLOPHRYS XAMI* (LYCAENIDAE)?

Additional key words: copulation, female remating, mating system.

Recent developments in sexual selection theory suggest that the role of females in shaping the evolution of mating systems has been underestimated (Eberhard 1996). In particular, female mating frequency is considered a variable that affects the potential for, and the strength of, sperm competition (Drummond 1984) and cryptic female choice (Eberhard 1985, 1996). In Lepidoptera, spermatophore counts have been used to determine the number of times a female has mated (Burns 1968, Drummond 1984, Eberhard 1985). However, the validity of spermatophore number as a measure of female mating frequency is based upon a number of assumptions which need to be verified before inferences about mating systems are made (Burns 1968, Lederhouse et al. 1989, Braby 1996). Here, I report the number of spermatophores found in a field sample of females of the lycaenid butterfly *Callophrys xami* (Reakirt); and, based upon previous information on the mating behavior and spermatophore production patterns in this butterfly (Cordero 1993, 1998), I discuss possible biases incurred when using such a measure as an estimate of female mating frequency.

I sampled females during eight sunny days, between 28 December 1989, and 23 January 1990 (this multivoltine species reaches its highest density between October and January (Soberón et al. 1988)) in the Pedregal de San Angel ecological reserve, located in the south of Mexico City (description of the area in Soberón et al. 1988). All females observed during these days were collected and frozen until dissection. I measured the length of the right forewing of each female with a calliper. I used this length as a measure of body size, considering that there was a positive correlation between wing length and body weight in a laboratory-reared sample of females ($r = 0.8$, $p < .001$, $n = 27$). I also determined the degree of female wing wear using the following scale: (1) similar to a recently emerged adult (wings mostly green on the ventral side with intact margins), (3) very worn female (wings mostly brown on the ventral side with worn margins), and (2) all individuals intermediate between (1) and (3). I evaluated the frequency of "successful" copulations by females (i.e., copulations that resulted in spermatophore transfer) by counting the number of spermatophores and spermatophore remains in the corpus bursae.

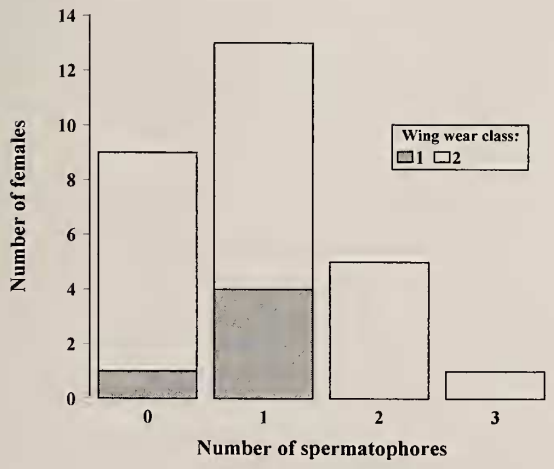

FIG. 1. Distribution of females with different number of spermatophores and the relationship between number of spermatophores and wing wear category.

The number of spermatophores in the 28 females I collected ranged from 0 to 3 (Fig. 1). The percentage of females without spermatophores was 32.1%, with one spermatophore was 46.4%, and with more than one spermatophore was 21.4%. The mean ± SD number of spermatophores found in females with at least one spermatophore was 1.37 ± 0.6. I looked for a relation between female wing length and spermatophore number with Spearman correlation because wing length was not normally distributed. This correlation was not significant ($r_s = -0.07$, $p > 0.05$, $n = 27$). Since all females collected were in wing wear conditions 1 or 2, I compared the number of spermatophores of females in each condition with a Mann-Whitney U test without finding significant differences ($U = 54$, $p > 0.05$; Fig. 1).

Since the work of Drummond (1984) is the only extensive summary presenting data from Lycaenidae, I use the data in that paper as a reference. Considering average number of spermatophores per mated female, maximum number of spermatophores, and proportion of females multiply mated, female Lycaenidae relative to other lepidopterans show the lowest degree of polyandry, comparable only with the Satyrinae (Drummond 1984). However, *C. xami* shows some differences when compared with the four lycaenid species included in Drummond (1984). The average copulation frequency estimated for mated females (1.37 ± 0.6), the maximum number of spermatophores (3), and the proportion of females multiply mated (21.4%) in *C. xami* is higher than in the other four lycaenids (ranges of average copulation frequency values: 1.05–1.17; maximum number of spermatophores: 2 in the four species; and proportion of females multiply mated: 3.7–12.7%). Furthermore, average number of copulations, maximum number of spermatophores, and proportion of females multiply mated could be underestimated in *C. xami*, since no females in the very worn ("old") wing wear condition were collected (Fig. 1). A sampling bias could exist if "old" females were more difficult to detect or to capture. However, our research group has been studying this species in the field for more than 10 years, and we have no evidence of any greater difficulty in observing and catching "old" females. It is possible that most females do not live long enough to become very worn and, therefore, are rare; in this case our estimates of copulation frequency would be unbiased. On the other hand, since outside the sampling period we have observed very worn *C. xami* females in the field, it is also possible that the abundance of "old" females varies in time as a result of, for example, varying predation pressure or weather conditions. Under these conditions, average and maximum number of spermatophores could vary with time depending on the age structure of females.

The method used to evaluate female copulation frequency in the field is based on three assumptions (modified from Drummond 1984):

Copulation always results in spermatophore transfer. In *C. xami* this is not true because there are some copulations of very short duration that do not result in the transfer of a spermatophore (Cordero 1993, 1998). However, these "interrupted" copulations are not common in the field (0/18 copulations observed in 1983–1985 and 2/27 copulations observed in 1989–1990; Cordero 1993). On the other hand, although the existence of interrupted copulations prevented the estimation of the total number of copulations performed by females, the figures obtained could be good estimates of the number of copulations resulting in spermatophore transfer.

Males transfer only one spermatophore per copulation. In *C. xami* this is not true since in laboratory experiments we observed three copulations in which different males transferred two spermatophores during one copulation (Cordero 1998). Violation of this assumption results in an overestimation of copulation frequency.

However, if the frequency of copulations resulting in the transfer of two spermatophores in the laboratory is a good estimate of their frequency in the field (3/199 copulations observed in the laboratory), its quantitative effect should be small.

Spermatophores always leave recognizable remains within the corpus bursae of the female. This is not true in *C. xami* since in the laboratory it was not always possible to observe clear spermatophore remains in very old females that had laid most of their eggs (pers. obs.). However, judging from wing wear, no female in this condition was sampled (see paragraph four above).

In conclusion, the possible violation of the first and the last assumptions, and the fact that some of the females may have mated again had they not been collected, results in an underestimation of the frequency of copulations in females; whereas the fact that some males transfer more than one spermatophore in one copulation results in an overestimation of the number of copulations. However, judging from the low frequency of "interrupted" copulations (4.4%), very worn females in the field (at least during the sampling period), and copulations resulting in the transfer of two spermatophores (1.5%), I conclude that spermatophore counts are a reasonably good estimate of female copulation frequency in *C. xami*.

ACKNOWLEDGMENTS

I thank Gabriela Jiménez and Dr. Rogelio Macías for their valuable technical help, and Dr. J. M. Burns and an anonymous reviewer for their comments. This research was supported by a Consejo Nacional de Ciencia y Tecnología (México) scholarship.

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Received for publication 5 April 1999; revised and accepted 16 December 1999.

Journal of the Lepidopterists' Society
53(4), 1999, 170–172

ADDITIONAL NOTES ON *PROSERPINUS CLARKIAE* AND *ARCTONOTUS LUCIDUS* (SPHINGIDAE) LIFE HISTORIES FROM THE PACIFIC COAST OF NORTH AMERICA

Additional key words: Onagraceae, Rubiaceae, *Gayophytum, Galium, Clarkia breweri, Clarkia modesta, Camissonia.*

Host associations for *Proserpinus clarkiae* (Boisduval) and *Arctonotus lucidus* (Boisduval) have recently been documented. *Proserpinus clarkiae* was found using *Clarkia unguiculata* (Lindley) in nature (Osborne 1995). Here, I compare results of my life history work on *P. clarkiae* with other results (Hardy 1959) on this species. The life history of *A. lucidus* is also known (Comstock & Henne 1942). However, the first natural host associations for *A. lucidus* were made by photographs and collections from *Clarkia* species in California, and are presented here along with observations on captive rearing of this moth. The immature stages of these related sphingid species have been confused in the field by some, possibly due to their sympatry, common use of *Clarkia* hosts, and superficial resemblance. Thus, I will also discuss morphological differences among these and other sympatric *Clarkia* feeding sphingids.

In presenting the biology of *P. clarkiae* (Osborne 1995), I repeated the assertion made by Hodges (1971) that its life history was unknown. Since that time, Dr. Frederick Rindge (American Museum of Natural History) has drawn my attention to a life history of *P. clarkiae* that predates both works. Larvae and a pupa reared from Vancouver Island (Hardy 1959) were described by Hardy (1959), and match the immatures of *P. clarkiae* from California. Hardy obtained seven ova by confining females over potted *Galium aparine* (Lewis & Szweykowski) (Rubiaceae). He reared at least one individual to pupation on that plant, but a field host was not given. The single fifth instar larva of *P. clarkiae* from Vancouver Island had the lateral dark blotches contiguous in an undulating line, a trait consistent with some (< 5%) of the California material I reared (most California larvae had oblique blotches disjunct) (Osborne 1995). This dark form may be typical of cool, wet, north coastal localities, where darker maculation may impart local selective advantages, or may be an artifact of captive rearing.

Dr. Robert Raguso, who studied sphingid pollination of *Clarkia* species in central California (see Raguso & Pichersky 1995, Raguso et al. 1996, Raguso & Light 1998), sent me several suspected *Proserpinus* larvae, a reared pupa, and a photograph (Fig. 1) of a fifth instar larva in nature on *Clarkia breweri* (A. Gray) E. Greene. These specimens were all collected from *C. breweri* and *Clarkia modesta* (Jepson) at Del Puerto Canyon, Stanislaus Co., California in May, 1991. However, instead of *P. clarkiae*, all were determined (by KHO) to be *Arctonotus lucidus*, a closely related species from a monotypic genus. Early instar *A. lucidus* larvae may be separated from *P. clarkiae* by the presence of a black anal horn which is absent in *P. clarkiae*. Fifth instar *A. lucidus* lose the anal horn, but have dorsal and lateral markings of olive green (but briefly black just after molt [Comstock & Henne 1942]), not black or gray as in *P. clarkiae*. In addition, *A. lucidus* can be distinguished from *P. clarkiae* on the basis of dorsal, transverse intersegmental lines of tan or cream breaking the olive green field, and ventral whitish or gray. The ground color in fifth instar *A. lucidus* larvae is variable (Comstock & Henne 1942), ranging from black to olivacious green to light green, to pink (Comstock & Henne 1942; D. Rubinoff pers. comm.; K. H. Osborne unpubl. obs.).